

Phenotypic Diversity among Croatian Common Bean (*Phaseolus vulgaris* L.) Landraces

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Summary

Phenotypic diversity among Croatian common bean (*Phaseolus vulgaris* L.) landraces was assessed by analysing 12 qualitative and six quantitative traits in 338 accessions collected from all production areas in Croatia. Out of 12 qualitative morphological traits, seed coat colour was the most polymorphic among accessions having the highest Shannon's information index and discriminating power. Out of six quantitative traits, the highest coefficient of variation was detected in duration of flowering. A weak correlation was found between distance matrices among accessions based on qualitative and quantitative traits.

Key words

common bean, landraces, morphological diversity, qualitative traits, quantitative traits, *Phaseolus vulgaris* L.

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Introduction

Common bean (*Phaseolus vulgaris* L.) is a widely grown grain legume crop with global agricultural and dietary importance. It demonstrates a global adaptability and phenotypic diversity. It is believed that cultivated common bean originates from the wild species *Phaseolus aborigineus* Burk. Two independent centres of domestication were identified: Mesoamerican (from northern Mexico to Colombia) and Andean (from southern Peru to north-western Argentina) (Gepts et al., 1986). Following the discovery of Americas, the common bean was transferred to Spain from where it was spread to Europe, Africa and Asia (Lešić et al., 2002).

Common bean is a food crop of a high nutritive value, consumed mostly as a mature dry grain but also as immature pods. Common bean seed is a valuable source of high quality proteins, carbohydrates, vitamins, minerals, dietary fibre, phytonutrients (flavonoids, lignins, phytosterols) and antioxidants (Reynoso-Camacho et al., 2006; Cardador-Martínez et al., 2002). Many of those compounds have important beneficial effects on human health (Duranti, 2006).

In Croatia common bean is a traditional grain legume, grown by small farmers using traditional agricultural techniques in low input production systems. Croatian traditional landraces are adapted to the specific growing conditions and agro-environments showing a great morphological diversity. The common bean is primary characterized by a high variability in seed traits such as seed shape, size and colour. The aim of this research was to analyse morphological traits of the traditional common bean landraces.

Materials and methods

Plant material

The plant material consisted of 338 common bean landraces collected from all the production areas in Croatia and included into the Grain legume collection of the University of Zagreb, Faculty of Agriculture, Department of Seed Science and Technology. The field trial was set up in Maksimir, Zagreb, in 2014.

Morphological analysis

Morphological analysis was carried out using the descriptor list for common bean used in Phaseolus Database (<http://www.genbank.at/en/ecpgr-phaseolus.html>). The accessions of common bean landraces were described based on 12 qualitative (QL) and six quantitative (QN) traits: flower colour of standard (vexillum) (QL01), flower colour of wings (QL02), plant growth habit (QL03), pods position (QL04), seed coat colour (QL05), seed pattern colour (QL06), character of pattern (QL07), seed brilliance (QL08), seed shape - longitudinal (QL09), seed shape - cross (QL10), colour of hilum (QL11), colour of hilum ring (QL12), number of days from sowing to flowering (QN1), duration of flowering (QN2), seed length (mm) (QN3), seed width (mm) (QN4), seed height (mm) (QN5), and weight of 100 seeds (g) (QN6).

Data analysis

Qualitative traits

Shannon's information index (H ; Shannon, 1949) as a measure of qualitative morphological trait diversity across common bean accessions was calculated for each trait as follows:

$$H_j = -\sum_{i=1}^I p_i \log_2 p_i$$

where p_i is the frequency of the i^{th} state and I is a total number of states of the j^{th} trait (Lewontin, 1972).

To compare the efficiency of the morphological traits in accession identification, the confusion probability (C) and discriminating power (D) of each trait were estimated following the approach developed by Tessier et al. (1999) for molecular marker data. Confusion probability (C) of the j^{th} morphological trait is thus calculated as follows:

$$C_j = \sum_{i=1}^I p_i \frac{(Np_i - 1)}{N - 1}$$

where N is the sample size. The confusion probability of a given morphological trait provides an estimate of the probability that two randomly chosen accessions from the sample of N accessions share identical states for a given morphological trait.

The number of indistinguishable pairs of accessions is equal to:

$$P_j = \frac{N(N-1)}{2} C_j$$

Thus, discriminating power (D_j) of the j^{th} morphological trait is equal to:

$$D_j = 1 - C_j = 1 - \sum_{i=1}^I p_i \frac{(Np_i - 1)}{N - 1}$$

denoting the proportion of distinguishable pairs of accessions.

Phenotypic dissimilarity between pairs of bean accessions was calculated using the proportion-of-shared-alleles distance (Bowcock et al., 1994) as implemented in MICROSAT (Minch, 1997). In this case, the results obtained by using the proportion-of-shared-alleles distance formula were identical to those obtained by 1 - simple matching coefficient: $D_{SM} = 1 - S_{SM} = 1 - (m / n)$, where m is the number of morphological traits shared between a pair of accessions and n is the total number of traits.

Quantitative traits

Pearson's correlation coefficients were calculated between the six quantitative traits using the SAS software PROC CORR procedure (SAS Institute, 2004). Principal Component Analysis (PCA) was applied in order to examine the interrelationships between common bean landraces and their quantitative traits using command PROC PRINCOMP in SAS program. The biplot was constructed by two principal PCs showing accessions and quantitative traits (as vectors). The standardized scores of the first three principal components (eigenvalue >1) were multiplied by the square root of their eigenvalues, and the pairwise Euclidean distance between accessions based on quantitative traits was calculated using the SAS software PROC DISTANCE. Distance matrices between accessions based on qualitative and

Table 1. Diversity of 12 qualitative morphological traits in 338 Croatian common bean accessions

| No. | Trait | Abbr. | <i>i</i> | <i>H</i> | <i>D</i> |
|-----|--------------------------------------|-------|----------|----------|----------|
| 1 | Flower colour of standard (vexillum) | QL01 | 5 | 1.174 | 0.504 |
| 2 | Flower colour of wings | QL02 | 5 | 1.167 | 0.500 |
| 3 | Plant growth habit (type) | QL03 | 2 | 0.996 | 0.499 |
| 4 | Pods position | QL04 | 5 | 1.926 | 0.706 |
| 5 | Seed coat colour | QL05 | 19 | 2.945 | 0.815 |
| 6 | Seed pattern colour | QL06 | 17 | 2.504 | 0.730 |
| 7 | Character of pattern | QL07 | 10 | 1.960 | 0.662 |
| 8 | Seed brilliance | QL08 | 3 | 0.831 | 0.300 |
| 9 | Seed shape - longitudinal | QL09 | 2 | 0.589 | 0.244 |
| 10 | Seed shape - cross | QL10 | 5 | 0.873 | 0.295 |
| 11 | Colour of hilum | QL11 | 9 | 1.716 | 0.543 |
| 12 | Colour of hilum ring | QL12 | 1 | –* | –* |

i - number of categories of trait; *H* - Shannon's information index; *D* - discriminating power; * - monomorphic trait

Table 2. Diversity of six quantitative traits in 338 Croatian common bean accessions

| No. | Trait | Abbr. | Average | SD | CV | Min | Max |
|-----|--------------------------|-------|---------|-------|-------|-------|--------|
| 1 | No. of days to flowering | QN1 | 58.81 | 7.27 | 12.36 | 49.00 | 76.00 |
| 2 | Duration of flowering | QN2 | 22.64 | 6.54 | 28.89 | 7.00 | 37.00 |
| 3 | Seed length (mm) | QN3 | 14.46 | 2.16 | 14.95 | 7.75 | 20.51 |
| 4 | Seed width (mm) | QN4 | 8.20 | 1.05 | 12.81 | 5.51 | 12.96 |
| 5 | Seed height (mm) | QN5 | 6.19 | 0.86 | 13.90 | 4.26 | 8.62 |
| 6 | Weight of 100 seeds (g) | QN6 | 48.51 | 13.15 | 27.10 | 13.18 | 110.10 |

SD - standard deviation; CV - coefficient of variation

quantitative traits were compared by calculating the correlation coefficient and implementation Mantel's test (Mantel, 1967). Mantel's nonparametric test was conducted based on 1,000 permutations using NTSYS-pc (Rohlf, 2005).

Results and discussion

Qualitative traits

Analysis of 338 common bean landraces revealed high phenotypic diversity. The number of categories (*i*), Shannon's information index (*H*) and discriminating power (*D*) of each qualitative trait are shown in Table 1. All the traits were found polymorphic except QL12 Colour of hilum ring. The highest number of categories (*i* = 19) as well as the highest Shannon's information index (*H* = 1.945) and discriminating power (*D* = 0.815) were detected for QL05 Seed coat colour. A high discriminating power (*D* > 0.600) was also detected for traits QL06 Seed pattern colour, QL04 Pods position and QL07 Character of pattern. These results coincide with those reported by Piergiovanni et al. (2000), Pereira et al. (2009) and Scarano et al. (2014).

The average distance between 338 common bean accessions based on 11 qualitative traits was $D_{SM} = 0.527$, ranging from 0.000 (accessions matching in all traits) to 1.000 (accessions

differing in all traits). From the total of 56,953 pairs of accessions, the number of indistinguishable ones was 520 (0.913%). The number of pairs of accessions that differed in all the traits was 307 (0.539%).

Quantitative traits

Table 2. shows the descriptive statistical parameters of six quantitative traits. The highest coefficient of variation was detected for the trait QN2 Duration of flowering (28.89), while the lowest f was detected for the trait QN1 Number of days from sowing to flowering (12.36).

A few of the six quantitative traits appeared to be highly intercorrelated (Table 3). A strong positive correlation was revealed between QN4 Seed width and QN6 Weight of 100 seeds ($r = 0.769$) and a moderate positive correlation between QN3 Seed length and QN4 Seed width ($r = 0.605$) as well as between QN3 Seed length and QN6 Weight of 100 seeds ($r = 0.672$).

Principal component analysis (PCA) based on six quantitative traits revealed that first three components accounted for 82.16% of the total variance. The first and second principal components had eigenvalues greater than one, while the largest difference was determined between the first and second eigenvalues. The correlations between the first three principal components

Table 3. Correlation coefficients (*r*; below diagonal) and their significance (*P*; above the diagonal) between six quantitative traits in 338 Croatian common bean accessions

| No. | Trait | Abbr. | Trait | | | | | |
|-----|--------------------------|-------|--------|--------|-------|-------|-------|-----|
| | | | QN1 | QN2 | QN3 | QN4 | QN5 | QN6 |
| 1 | No. of days to flowering | QN1 | | *** | * | *** | ns | *** |
| 2 | Duration of flowering | QN2 | 0.332 | | *** | *** | * | *** |
| 3 | Seed length (mm) | QN3 | 0.139 | 0.250 | | *** | ns | *** |
| 4 | Seed width (mm) | QN4 | 0.259 | 0.243 | 0.605 | | *** | *** |
| 5 | Seed height (mm) | QN5 | -0.035 | -0.111 | 0.027 | 0.460 | | *** |
| 6 | Weight of 100 seeds (g) | QN6 | 0.213 | 0.182 | 0.672 | 0.769 | 0.445 | |

*** $P < 0.001$; ** $0.001 < P < 0.01$; * $0.01 < P < 0.05$; ns $P > 0.05$ **Table 4.** Pearson's correlation coefficient between six quantitative traits and values of the first three main components

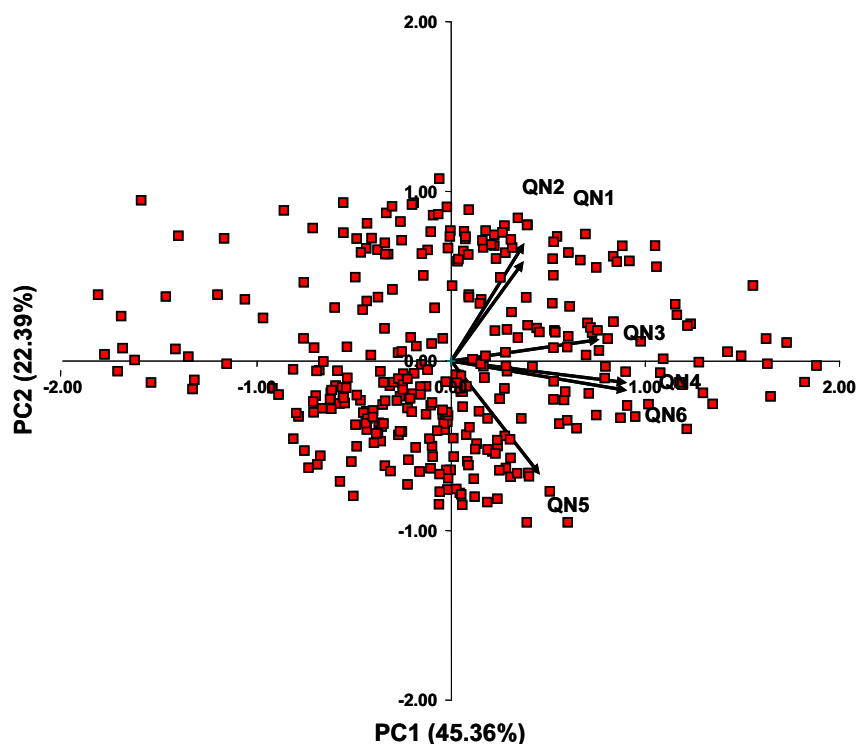
| No. | Trait | Abbr. | Principal component | | |
|------------------|--------------------------|-------|---------------------|------------|------------|
| | | | PC1 | PC2 | PC3 |
| 1 | No. of days to flowering | QN1 | 0.374 *** | 0.589 *** | 0.562 *** |
| 2 | Duration of flowering | QN2 | 0.378 *** | 0.695 *** | 0.080 ns |
| 3 | Seed length (mm) | QN3 | 0.765 *** | 0.127 * | -0.543 *** |
| 4 | Seed width (mm) | QN4 | 0.906 *** | -0.128 * | 0.033 ns |
| 5 | Seed height (mm) | QN5 | 0.457 *** | -0.671 *** | 0.489 *** |
| 6 | Weight of 100 seeds (g) | QN6 | 0.908 *** | -0.174 ** | -0.086 ns |
| Eigenvalue | | | 2.722 | 1.343 | 0.864 |
| % Total variance | | | 45.36 | 22.39 | 14.41 |

*** $P < 0.001$; ** $0.001 < P < 0.01$; * $0.01 < P < 0.05$; ns $P > 0.05$

and six quantitative traits are shown in Table 4. The biplot was constructed by the first two PCs showing common bean landraces and six quantitative traits (as vectors) (Fig. 1).

Three out of six traits showed a strong positive correlation with the first principal component which explained 45.36% of the total variance (QN6 Weight of 100 seeds, QN4 Seed width, and QN3 Seed length). The second principal component accounting for 22.39% of the total variance showed a moderate positive correlation with QN2 Duration of flowering and QN1 No. of days to flowering and a moderate negative correlation with QN5 Seed height (Table 4).

The average Euclidean distance between 338 analysed accessions based on six qualitative traits ranged from 0.009 to 12.279, with an average value of $D_{EUC} = 2.486$. The correlation coefficient between distance matrices among accessions based on qualitative and quantitative traits was $r = 0.393$ ($P_{Mantel} < 0.0001$), after 10.000 permutations of Mantel's test.

Figure 1. Biplot obtained by principal component analysis on the basis of six quantitative traits in 338 Croatian common bean accessions

Conclusion

In Croatia common bean is traditional but neglected grain legume, grown by small farmers using traditional agricultural practices in low input production systems. Croatian landraces are adapted to specific growing conditions showing a great morphological variability.

Phenotypic diversity among Croatian common bean (*Phaseolus vulgaris* L.) landraces was assessed by analysing 12 qualitative and six quantitative traits in 338 accessions collected from all production areas in Croatia.

Out of 12 qualitative morphological traits, the trait QL5 Seed coat colour was the most polymorphic among accessions having the highest number of categories as well as the highest Shannon's information index and discriminating power, while the trait QL12 Colour of hilum ring was found monomorphic.

Out of six quantitative traits, the highest coefficient of variation was detected for the trait QN2 Duration of flowering. A strong positive correlation was revealed between the trait QN4 Seed width and QN6 Weight of 100 seeds.

Principal component analysis based on six quantitative traits revealed that the first two principal components had eigenvalues greater than one, jointly explaining 67.75% of the total variance. Traits related to the first principal component were QN6 Weight of 100 seeds, QN4 Seed width and QN3 Seed length, while QN2 Duration of flowering, QN1 Number of days to flowering and QN5 Seed height were related to the second principal component.

The correlation coefficient between distance matrices among accessions based on qualitative and quantitative traits was highly significant, but weak.

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